SYNOPSIS

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Subject : Zoology

Entitled :- **Study of effect of selected non steroidal Anti-inflammatory Drugs on *Cyprinus carpio* (Linnaeus,1758)**

STUDY OF EFFECT OF SELECTED NON STEROIDAL & ANTI INFLAMMATORY DRUGS ON CYPRINUS CARPIO (LINNAEUS ; 1758)

INTRODUCTION :

At the present rate of urbanization and modernization, the human society is consuming increasing amounts of medicinal drugs in general and non-steroidal & anti-inflamatory drugs (NSAIDs) in particular. A rapidly growing body of scientific and research documentation indicates that a number of substances have the potential to interfere with normal functions of the body in animals.

(NSAIDs) are substances in our surroundings, food, consumer products which interfere with hormone biosynthesis, metabolism, or action resulting in a disturbance of normal homeostatic control of vital and other physiological processes in animals.

Accumulating evidence clearly indicates that (NSAIDs) have affects on general metabolism. Results from animal experimentation, clinical observations in human and other sources implicate (NSAIDs) as a significant concern to environmental and human health.

Research both at national and international level has seen study and experimentation on many different animal models. Most of the research done so far has focused on mammalian models, especially rodent models, owing to the possibility of direct correlation of such studies with higher mammals. But in the interest of conservation of animal biodiversity more research in non-mammalian animal models is necessary to enrich our understanding of the impact of these chemicals on the different organisms in the environment.

OBJECTIVES OFTHE STUDY :

There are gaps in our knowledge of the methodologies for analysis, exposures and effects of the (NSAIDs). Therefore, new information and activities relevant to these substances are rapidly emerging. This effort calls for effective coordination of research, testing, assessment and sound management of the substances.

Further work is needed in a number of areas to help us gain better insight into the effects of (NSAIDs) on wildlife populations and to develop and validate novel whole organism assays for endocrine-disruption in non-mammalian vertebrates like fish,amphibians, reptiles and birds, both to assess hazards to those speciesand to evaluate long term environmental effects.

Field studies are required where NSAID effects are suspected; these should involve a broadly based screen including assessment of gonadal function, behavioral patterns and offspring sex ratio, numbers and survival. Generally, environmental toxicity studies should focus on aquatic organisms as environmental exposure to industrial chemicals generally involves discharges to sewage. It is exposure assessment studies like this one that provide with the toxicity data required for conservation of organisms likely to be exposed and management of the chemicals involved.

Proposed Studies:

About **Cyprinus carpio** (Linnaeus, 1758)

Cyprinus carpio is a fish inhabiting warm, deep, slow flowing and still waters such as lowland rivers and large well

vegetated lakes. Introduces in all types of water bodies. This fish spawns along shore in back waters. Larvaldevelopment occurs in very warm waters, along shallow submerged vegetation.

It is omnivorus feeding on a diet of water plants but prefer to scavenge the bottom for insects, crustaceans (zooplankton), crow fish, benthoic worms. It can grow to a maximum length of 120 cm **(citation ???)**. Members of genus Cyprinus are widely consumed in lower and middle class economic group. Now it is contributed much more to the fishery economy in India as compared to china.

The present work proposes to study the effects of aquatic contaminants - NSAIDs - Diclofenac & Ibuprofen on general biology and fin regeneration of Cyprinus carpio.

About **DICLOFENAC :**

Diclofenac is a non steroidal anti inflammatory drug and is regularly detected in surface water. It is used for treatment of painful and dysmorhaea inflammatory conditions, rhemoitoid arthritis, spondylites, kidney stone, gal stone and acute migrains. It was first synthesized by Alfred Sallmann and Rudalf Pfister and introduced to Voltaren Cibagiegy (now Novartis) in 1973. It may be supplied with sodium (Na) or Pottasium (K) salts.

The estimated overall worldwide consumption of DCF as a human and veterinary pharmaceutical drug is >1000 tons per year (Zhang Y. et al. 2008). It combined with opinoids if need combination of DCF and codeine.

Use of DCF entails vascular and coronary risk, upper gastro intestinal complications and vascular deaths also reported.

Research results upto April 2006, increased rate of heart disease 1.63 compared to non users.

Acute testing resulted in relatively low toxicity of DCF for fish, daphnia & algae. **With no observed effect concentration (NOEC)** (Cleuvers M. et al. 2003; Lee J. et al 2011). However upon chronic exposure several authors observed histopathological effects in trout sp. at relatively low concentrations around 1 to 5μg/L (Schawaiger J. et al. 2004; Mehinto A.C. et al 2010). These finding induced a public concern regarding a risk of DCF to fish population.

The seeming high octanol-water partition coefficient **kow** of DCF (Avdeef A et al 1998) also called concern regarding high bioconcentration in fish & subsequent potential for secondary poisoning within aquatic food chain and to fish eating birds. A significant bioconcentration of DCF was measured in some fish organs (Schwaiger J. et al). The warm water fish *Daniorerio* was tested in early life stage with long term exposures studies, with DCF by following Organization for Economic Cooperation and Development (OECD) test guidelines 210. Histopathology of potentially targeted organs (Kidney, liver and gills) was additionally conducted on *Onchorynchuss mykiss* to verify earlier pathological findings and adverse biological effects to development, growth and survival of fish. Additionally, the bioconcentration of DCF was studying in *O. mykiss* by using internationally accepted OECD test guidelines 305 (citation).

About **IBUPROFEN :**

Ibuprofen is one of the NSAIDs and widely used for analgesic, antipyretic and anti-inflammatory purposes to relieve symptoms of arthritis, rheumatoid disorders and fever. (Hayashi et al. 2008), so it is included in “Essential drug list” of WHO. Therefore is Ibuprofen manufactured in large quantities (Heckman et al 2007). It was discovered in 1961 by Stewards Adams & marketed as Brufen.

Ibuprofen has frequently been detected in surface water, with as much as 0.1 μg/L detected in surface water of South Wales and U.K. (Kasprazyk - Hordern et al., 2008). The average concentration detected in major rivers of Korea was 0.03 μg/L (Kim et al 2007). Relatively greater concentration of Ibuprofen have been reported for effluents (upto 22 μg/L) and ifulents (upto 84 μg/L) of sewage treatment plants (STPs) (Brun et al 2006; Gomez et al 2007). Hence municipal waste water of effluents are an important source of ibuprofen in aquatic environments specially streams and rivers.

Due to widespread occurrence of Ibuprofen in aqueous environments and its potential for ecological impact has been of growing concern (Christensen et al 2009). Ibuprofen in known to influence cyclo-oxygenase (COX) pathway (Flippin et al 2007; Haeckmann et al 2008). Therefore Ibuprofen contamination could affect reproduction of aquatic animals i.e. indirectly on skin and fins. Ibuprofen effects the heart burn and rash, gastrointestinal bleeding. It increases risk of heart failure, kidney failure and liver failure. It is weaker than other NSAIDs.

It is used in mild to moderate pain (including pain relief after surgery), dental pain, headache and pain from kidney stone, also painful in menstruation.

Study Parameters:

The present study aims to investigate the effect of selected NSAIDs on the fish:

- Experimental determination of the effects of diclofenac and ibuprofen on Cyprinus carpio.
- Effects on respiration.
- Reproductive aspects like fecundity & mating behavior.
- Biochemical composition - protein, carbohydrate, lipid content in muscles, gills and kidney.
- Hematological parameters - like RBC and WBC counts.

The experimental methods of investigation in this work would be conducted in the context of the dynamic nature of the physico-chemical properties of water- a medium in which the subject of this investigation inhabits.

The fish would be acclimatized to laboratory conditions and experiments would be conducted subsequently. A consistent supply of fish for experimentation is possible from the fishermen and fish farms in and around Nanded district.

This study is broken into the following **chapters :**

1. Respiratory Metabolism
2. Reproduction
3. Hematology
4. Histology
5. Regeneration

Exposure to selected chemicals would evaluated in relation to the physico-chemicals properties of water, e.g. temperature, light intensity & pH.

Respiratory metabolism:

Rate of oxygen consumption, total oxygen consumption would be determined by exposing the fish to the said chemicals for certain duration of time. These determinations would be carried out by the standard Winkler's method. The effect of temperature of oxygen consumption of fish before and after exposure to the selected chemicals would also be explored.

Reproduction:

Fecundity, secondary sexual characters like coloration, mating behavior, total fecundity and realized fecundity estimations would be made by extracting the ovary from the fish. These estimations would also be performed at different exposure times to the chemicals.

Effect of the chemicals on the anatomy and other physical features of the reproductive system of the fish would also be studied. It is well known that temperature has a noticeable effect on the coloration of fish. This aspect of temperature dependence of coloration would be investigated in the context of exposure to the selected chemicals; and effects of the chemicals would be evaluated in the context of environmental and reproductive health of the fish.

Biochemical composition:

Organic contents to be studied are total proteins, total carbohydrate, lipids. These estimations are to be carried out in tissues like liver, body muscle, gills, kidney etc.

Hematology:

Aspects like RBC and WBC counts, hematological changes are commonly investigated in animal experimental studies. Blood cell count is an important indicator of stress, especially physiological stress. RBC and WBC counts of the fish exposed to the different chemicals under different temperatures would be studied.

The experimental sets would be designed as per the size and age of the fish. Control and experimental groups would be exposed to different sublethal concentrations of the compounds for varying durations of time and then investigated for different parameters as stated above.

The present proposed study will be highly beneficial in understanding the effects of the different chemical contaminants on the aquatic ecosystem in general and fish in particular. The findings could be helpful in predicting the possible adverse outcomes of the long term exposure of humans to such contaminants.

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